American Journal of Hematology 64:32–38 (2000)

The Japanese Multicenter Open Randomized Trial of Ursodeoxycholic Acid Prophylaxis for Hepatic Veno-Occlusive Disease After Stem Cell Transplantation

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Hepatic veno-occlusive disease (VOD) is a common transplant-related complication of stem cell transplantation. There is no safe and proven therapy for established VOD, and attempts have focused on its prevention. Limited studies have suggested that prophylactic use of ursodeoxycholic acid (UDCA) reduced the incidence of VOD. To confirm the preventive effect of UDCA on VOD, we conducted a prospective, unblinded randomized, multicenter study of UDCA involving 132 patients who underwent stem cell transplantation for a variety of disorders. Sixty-seven patients were assigned to the UDCA-treated group, and 65 patients were assigned to the control group. The clinical characteristics of the two groups were similar with respect to primary diagnosis, age, sex, and baseline organ function. The preparative regimen and GVHD prophylaxis did not differ significantly between the two groups. UDCA was highly effective in preventing VOD, which occurred in only 3.0% in the UDCA-treated group, as opposed to 18.5% in the control group ($P = 0.0043$). There were no adverse effects attributable to UDCA. The initial promising report of a prophylactic effect of UDCA on VOD after stem cell transplantation was confirmed in this prospective study. Am. J. Hematol. 64:32–38, 2000.

Key words: hepatic VOD; prophylaxis; ursodeoxycholic acid (UDCA); stem cell transplantation

INTRODUCTION

Hepatic veno-occlusive disease (VOD) has been reported to occur in 10% to 50% of transplanted patients, with a mortality rate of up to 50% of all affected patients [1,2]. Various factors are identified as risk factors for the development of VOD: history of liver dysfunction, elevated AST level, decreased pseudocholinesterase level, transplant from an HLA-mismatched or unrelated donor, and abdominal irradiation, but no effective means of prevention has been firmly established [3–5]. Recently, prophylactic use of heparin, low molecular weight heparin, pentoxifylline, and prostaglandin E1 has been shown to be effective in the prevention of VOD [6–8]. These trial drugs have met with mixed success, and none of them has been universally accepted for prevention of the mortality associated with severe VOD. A nontoxic treatment that could reduce hepatic damage and cause VOD to be

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Received for publication 9 April 1999; Accepted 5 January 2000

manifested as reversible liver damage rather than as a progressive and fatal disease is strongly needed [9]. Ursodeoxycholic acid (UDCA) may fulfill this role. Recently, Essell et al. reported that UDCA effectively reduced the incidence of VOD without major adverse effects [10,11]. Their conclusions were based on results obtained in patients given busulfan and cyclophosphamide as conditioning therapy and may not apply to patients conditioned with other regimens. We undertook a randomized, prospective, multicenter study to determine whether UDCA could decrease the incidence of VOD after stem cell transplantation with a variety of conditioning chemoradiotherapies.

MATERIALS AND METHODS

Study Design

Between June 1996 and February 1998, 136 patients from nine transplant centers in Japan were enrolled for this study. Patients were randomized to receive or not to receive prophylactic UDCA. Seventy-one patients were allocated to the UDCA group, and 65 patients to the control group. According to the protocol, UDCA was administered orally from day −21 and until day 80 after transplantation. The daily dose of UDCA was 600 mg. An intent-to-treat policy was followed, and, accordingly, patients continued in the study irrespective of possible discontinuation of UDCA. Patients were monitored from the time of enrollment to 24 weeks after transplantation (median, 182 days). Written informed consent was obtained from all the patients before they were enrolled for the study.

VOD Evaluation

VOD was diagnosed when at least two of the following conditions occurred concomitantly after transplantation: jaundice (bilirubin level ≥2.0 mg/dL), painful hepatomegaly, and fluid accumulation evidenced by ascites or unexplained body weight gain (≥2% above base line weight). Imaging techniques such as ultrasonography and/or CT were also used to assess VOD.

Statistical Analysis

The main outcome measure of this study was the incidence of VOD. The control group was expected to show a 30% incidence of VOD, and a sample size of 62 patients in each group was required to detect a reduction in incidence to 10% in the UDCA-treatment group, with a type I error of 0.05 and a power of 80%. Categorical variables were compared using the χ^2 test or Fischer's exact test, and continuous variables were compared using the Wilcoxon 2-group test. A multivariate analysis was performed to determine the risk factors for the occurrence of VOD by the logistic regression model. All tests were two-sided. All analyses were done using JMP software (version 3.2; SAS Institute Inc, Cary, NC).

TABLE I. Clinical Characteristics*

Characteristics	UDCA group	Control group	Total
Patients (*N*)	67	65	132
Mean age (years)	34.5	35.7	35.1
Sex (*N*)			
Male/Female	40/27	30/35	70/62
Diagnosis (*N*)			
CML	15	15	30
ANLL	23	19	42
ALL	15	12	27
MDS	4	4	8
Lymphoma	3	8	11
AA	3	5	8
Other	4	2	6
Type of transplant (*N*)			
Allogeneic			
GI	29	27	56
PI	8	4	12
MR	2	0	2
UR	22	25	47
Autologous	6	9	15
Preparative regimen (*N*)			
Chemotherapy only			
BU/CY	17	11	28
BU/VP	1	1	2
TT/CY	1	0	1
MCNU/VP/CY	2	4	6
LPAM	2	1	3
other	0	1	1
Chemotherapy + TBI			
CA/CY/TBI	16	16	32
TT/CY/TBI	13	13	26
other	5	8	13
Chemotherapy + TLI			
BU/CY/TLI	7	6	13
CY/TLI	3	3	6
other	0	1	1
GVHD prophylaxis			
MTX/CSP	60	53	113
MTX/FK506	0	1	1
CSP only	1	2	3

*Abbreviations: GI, genotypically identical; PI, phenotypically identical; MR, mismatch related; UR, unrelated; BU, busulfan; CA, cytarabine; CY, cyclophosphamide; VP, etoposide; TT, thiotepa; TBI, total body irradiation; TLI, total lymphoid irradiation; MTX, methotrexate; CSP, cyclosporine.

RESULTS

Patients

One hundred thirty-six patients were enrolled for the study. Four patients were excluded: one who died due to regimen-related toxicity, unrelated to VOD, on the day of stem cell infusion, one who later canceled stem cell transplantation, and two whose data sheets were not retrieved. Therefore, 132 patients were included in the final data analysis. Of them, 67 patients had been assigned to the UDCA group, and 65 patients had been assigned to the control group. Detailed clinical characteristics of the study population are summarized in Table I. The pretransplant characteristics of each group were essentially

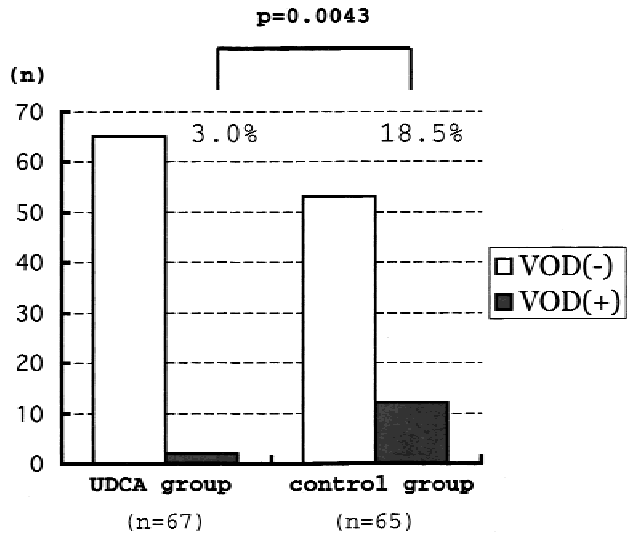

Fig. 1. Effects of UDCA on the incidence of VOD after stem cell transplantation. UDCA was found to be highly effective in preventing VOD. The incidence of VOD in the UDCA group is significantly lower than that in the control group (2 of 67 patients compared with 12 of 65 patients: *P* = 0.004).

similar with respect to age, sex, underlying disease, preparative regimen, type of graft, and graft-versus-host disease (GVHD) prophylaxis. No significant differences were detected between the two groups in preconditioning liver function test, hemoglobin, leukocyte count, or platelet count (data not shown), nor were there any significant differences between the two groups in terms of risk factors for VOD; namely, history of liver dysfunction and elevated AST level before transplantation. No patient had an active systemic fungal infection at the time of starting the preparative regimen. There were four cases of second transplantation (2 in the UDCA group and 2 in the control group).

Thirty of the 67 UDCA-treated patients had at least one episode of temporary interruption of UDCA therapy. The main reason for discontinuation of the drug was difficulty swallowing the capsules because of severe nausea during conditioning chemoradiotherapy and/or severe mucositis after transplantation. These 30 patients were included in the UDCA group in this intent-to-treat analysis to eliminate potential bias favoring the UDCA group.

Incidence of VOD

Fourteen (10.6%) of the 132 patients developed hepatic VOD. The incidence of VOD in the UDCA group was significantly lower than that in the control group: 2 patients (3%) and 12 patients (18.5%), respectively (Fig. 1). The clinical features of the 14 patients who developed VOD are summarized in Table II. Of the 14 patients in whom VOD was diagnosed, 13 met the criteria for VOD. One patient (UPN 102), who met only one of the criteria for VOD, was diagnosed as possibly having VOD. In this case, signs of VOD developed on day 50 and progressed. Serial abdominal ultrasound showed persistent right pleural effusion, mild hepatomegaly, and ascites until day 80. During this period, her bilirubin rose to 1.9 mg/dL. Abdominal angiography performed on day 70 revealed portal hypertension. Hepatic venous pressure gradient, measured at that time, was approximately 13 mmHg. Although histological confirmation was not possible due to lack of the special device for liver biopsy, this case was clinically diagnosed as VOD.

VOD occurred during the first month in 11 cases, and later in 3 cases. The latter 3 cases seemed to have late-onset type VOD, this situation has been recently addressed [12]. According to previously defined clinical criteria, the severity of VOD was mild to moderate in most cases. VOD resolved in all 14 patients, but 4 patients eventually died. The causes of death were relapse, GVHD, sepsis and bronchiolitis obliterans (1 case each); none of them died of hepatic failure. Two patients who developed VOD in the UDCA group had an episode of early discontinuation of UDCA therapy and did not take any UDCA after day 47 and day 5, respectively. It is especially noteworthy that no cases of VOD were observed in patients who could take UDCA for the entire prescribed period.

Influence of UDCA on Clinical Course

Although UDCA has been known to be associated with several minor adverse effects [13], no presumably drug-induced side effect was observed in our patients.

The time course of hepatic biochemical tests is illustrated in Figure 2. Mean serum ALP level was significantly lower in the UDCA group compared with the control group ($P = 0.021$). On the other hand, there was no significant difference between the two groups in regard to bilirubin, γ GTP (Fig. 2), AST, ALT, leukocytes count, hemoglobin, or platelet count (data not shown).

The incidence of GVHD and relapse was similar in both groups (data not shown). There were 15 deaths in the UDCA group, compared with 16 deaths in the control group. The causes of death were similar in both groups. Probability of survival as analyzed by the Kaplan–Meier plot, did not differ significantly between the two groups (data not shown).

Analysis of Risk Factors

The results of univariate and multivariate analyses of risk factors for the occurrence of VOD are shown in Table III. By univariate analysis, UDCA administration, pretransplant anemia, and high ALP values were significantly influenced to the incidence of VOD, whereas age, sex, primary diagnosis, and type of transplant did not reach statistical significance. A multivariate analysis with logistic regression model identified a busulfan-

TABLE II. Clinical Findings of Patients Who Developed VOD

UPN	Diag.	Type of trans-plant	Regimen	UDCA	Onset of VOD (day)	Painful hepato-megaly	Ascites	Greatest weight gain (%)	Highest bilirubin value (mg/dL)	Highest ALT value (IU/L)	Outcome
19	AML	UR	CACYTBI	No	16	No	No	6.7	3.6	67	Alive
20	MM	Auto	LPAM	No	12	No	Yes	16.7	3.0	58	Dead
43	CML (AP)	UR	TTCYTBI	No	26	Yes	Yes	5.0	44.9	145	Dead
51	AML	UR	CACYTBI	No	7	No	No	3.2	2.6	38	Alive
55	CML	GI	BUCYVPIDA	Yes	11	Yes	Yes	9.0	11.2	27	Dead
67	AML	GI	BUCYCA	Yes	43	Yes	Yes	11.8	11.7	395	Alive
73	CML	GI	BUCY	No	23	Yes	Yes	9.6	3.1	4265	Alive
77	AML	GI	BUCYCA	No	14	No	No	5.7	2.8	50	Dead
81	SAA	PI	BUCY	No	40	Yes	Yes	0.0	1.5	389	Alive
86	CML	GI	TTCYTBI	No	14	No	No	6.1	2.6	53	Alive
93	AML	GI	CACYTBI	No	10	No	No	12.0	3.3	27	Alive
109	ALL	UR	TTCYTBI	No	24	Yes	No	10.1	1.5	33	Alive
118	SAA	UR	CYTLI	No	7	No	No	14.6	4.0	80	Alive
120	AML	UR	BUCYTLI	No	50	No	Yes	9.6	1.9	68	Alive

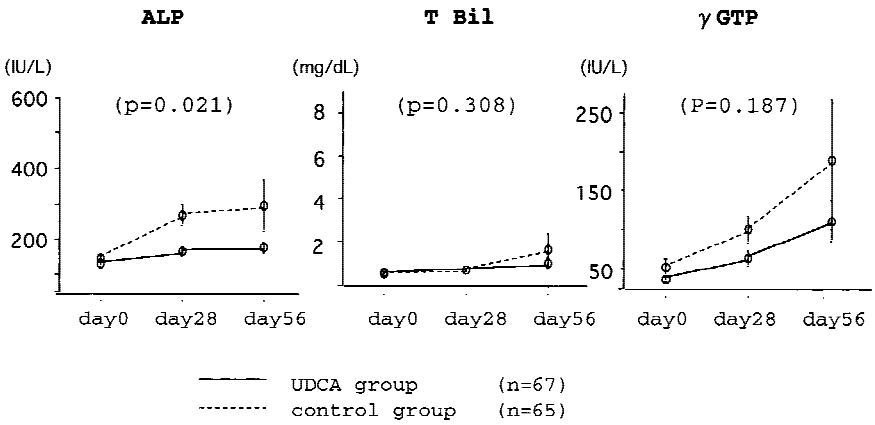

Fig. 2. Influence of UDCA treatment on the concentration of alkaline phosphatases, total bilirubin, and γ GTP. The UDCA group, *N* = 67, solid line; the control group, *N* = 65, dashed line. Data are shown as the mean ± S.E. Each *P* value is presented on the middle of a figure.

TABLE III. Univariate and Multivariate Analysis

	Univariate *P*	Multivariate *P*	Odds ratio (95% CI)
Age	0.9614	0.8778	1.05 (0.94–1.07)/10 years
Sex (Male/Female)	0.4141	0.5380	0.79 (0.38–1.66)
Type of transplant (Auto/Allo)	>0.999	0.6461	1.34 (0.38–4.73)
ALP prior conditioning	0.0062[a]	0.0040[a]	3.82 (1.53–9.53)/100 U
Hb prior conditioning	0.0229[a]	0.0290[a]	1.41 (1.03–1.93)/1g/dL
Busulfan containing-regimen	0.2305	0.0211[a]	2.54 (1.15–5.64)
UDCA (Yes/no)	0.0046[a]	0.0072[a]	3.39 (1.39–8.24)

[a]Statistically significant.

containing preparative regimen as a factor that significantly influenced the occurrence of VOD, in addition to the three independent variables identified by univariate analysis.

DISCUSSION

No appropriate treatment for patients with VOD has been established as yet. Early treatment with thrombolytic therapy showed promising results but this therapeutic modality was associated with a potential risk of serious bleeding complications [14]. Attempts to prevent VOD have been carried out by several investigators. Essell et al. reported that the incidence of hepatic VOD in 22 allogeneic BMT patients prophylactically treated with UDCA was 9.1% compared with 64.3% in 28 historical control patients [10]. The encouraging results of this pilot study were further supported by a randomized, double-blind, placebo-controlled trial [11], which showed that UDCA prophylaxis was highly effective in preventing

VOD (14.3% in the UDCA group versus 40.6% in the control group, $P = 0.02$). The satisfactory effect of UDCA on transplant recipients who had been conditioned with busulfan and cyclophosphamide raises the question of whether this medication would also be effective in transplant recipients treated with other conditioning regimens. The results of the present study, including 91 patients conditioned with other regimens such as TBI containing regimens, showed a lower incidence of VOD in patients who were treated with UDCA.

The multivariate analysis of the risk factors in this series confirmed the findings of some other published studies, but they differed in regard to other factors. Age and sex were not significantly associated with VOD. The conditioning regimen containing busulfan carried a high risk of VOD, but it was not found to be significant in the univariate analysis. An increased ALP value prior to conditioning therapy has never been described before as a risk factor for the occurrence of VOD. Anemia was also significantly associated with VOD, and this could be explained by the fact that anemic patients were mostly treated with high-dose chemotherapy for a long period, which is well known to be associated with a higher risk of VOD [15].

Our study provided evidence that prophylaxis with UDCA reduced VOD without any major adverse effects. It is unlikely that the use of UDCA simply decreased the mean bilirubin level, which would result in the apparent reduction of the incidence of VOD, since the time course of bilirubin values did not show any significant differences between the two groups (Fig. 2).

The low incidence of VOD in the UDCA group might be associated with the decreased absorption of busulfan [16,17]. To examine the influence of UDCA on busulfan absorption, plasma busulfan concentrations were pharmacokinetically monitored. Comparison of the UDCA group with the control group showed no significant difference in the mean busulfan $AUC_{0-360\ min}$ (685 ± 121 versus 510 ± 301 μM/min), as shown in Figure 3B.

The actual incidence (18.5%) of VOD in the control group in our study was less than the expected incidence (30%) and most of VOD cases were classified as mild to moderate compared to previously reported cases. Several factors may have contributed to these discrepancies. In our study, the mean age of the patients group was relatively young, which may partially explain the low incidence and very mild degree of VOD. If VOD occurred in 18% of patients undergoing stem cell transplantation, then each study group (control and UDCA) would have to include 65 patients to demonstrate a reduction in VOD to 3% (power, 0.8; $P = 0.05$). Therefore, the total number of patients ($N = 132$) included in our study had still enough power to show a significant difference in the incidence of VOD. In our study, no survival advantage was observed in the UDCA group. Another double-blind

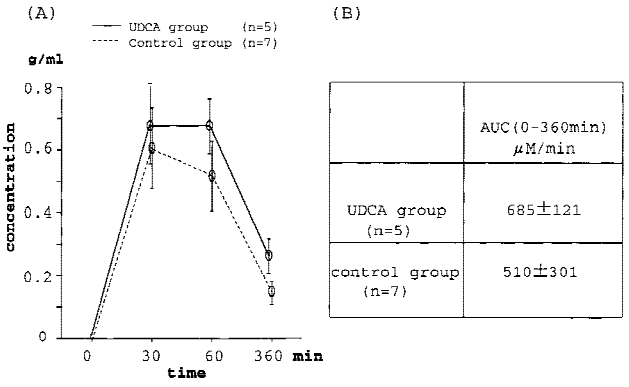

Fig. 3. Busulfan pharmacokinetics. Plasma busulfan concentrations were monitored pharmacokinetically. Plasma was collected from 12 patients (5 in the UDCA group, 7 in the control group). Busulfan concentrations were measured by high-performance liquid chromatography and the details are described elsewhere [16]. After the first dose at noon, blood was sampled at 0 min, 30 min, and 6 hr. (A) Busulfan concentration versus time. (B) The mean (±S.D.) busulfan $AUC_{0-360min}$ using the trapezoidal rule.

and placebo-controlled study also failed to demonstrate a survival advantage with UDCA [11]. Of note, however, is that a larger prospective study is required to show a statistically significant survival advantage with UDCA. If we assume that the therapy related mortality rate is about 30% after stem cell transplantation and an approximately 15% survival advantage is anticipated to UDCA treatment (power, 0.8), the required trial size would be 220 patients [18].

Endothelial cell (EC) damage, which triggers local thrombotic mechanisms, leading to microvascular flow insufficiency, production of cytotoxic substances, and ultimately hepatocellular necrosis, has been thought to be an early event in the development of VOD [19]. Recently, a direct prophylactic effect of bile steroids, including UDCA, on EC damage has been shown by Ishida et al. [20]. In a rat model of EC damage induced by lactic acid injection, the bile acid sodium scymnol exhibited a significant prophylactic effect [21]. Furthermore, several in vitro studies provide potential mechanisms whereby UDCA may attenuate the pathogenesis of VOD. UDCA can protect hepatocytes from ethanol induced damage by down-regulating the mRNA expression of inflammatory cytokine such as TNF α and IL-1 α [22]. These cytokines not only induce direct liver damage but also can be associated with apoptosis in EC [23]. Apoptosis is one cause of EC damage and thus contributes to development of VOD. Recent data have supported a novel function for UDCA in regulating programmed cell death. UDCA can inhibit the apoptosis induced by deoxycholic acid, TGF-1, and Fas ligand and protects against the membrane-damaging effects associated with hydrophobic bile acids in both hepatocytes and non-liver cells [24]. A possible explanation for the antiapoptotic effects of UDCA may

involve the mitochondrial membrane. UDCA has been shown to exert a protective effect against mitochondrial membrane permeability transitions promoted by various compounds, including hydrophobic bile salts [25]. In addition, UDCA may increase the glutathione content of hepatocytes, thereby exerting a protective effect on the development and progression of cytokine-induced damage [22]. Thus, it has become apparent that UDCA may exert a direct effect at the cellular, subcellular, and molecular levels, not only in hepatocytes but in cells of non-hepatic origin.

In summary, the results of our study demonstrate that UDCA has a beneficial effect on the prevention of VOD that is consistent with the findings of the previous randomized study performed by Essell et al. The mechanism of the cytoprotective effect of UDCA against chemotherapy and irradiation requires further elucidation, but the standard use of UDCA as a prophylaxis for VOD in transplant recipients who are treated with a chemo-irradiation regimen should be considered.

ACKNOWLEDGMENTS

We are indebted to Dr. H. Ishida, H. Akiyama, S. Mori, and K. Hiruma, for their critical review of the manuscript and many helpful suggestions.

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